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Natural Products for Pest Management

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Chapter 16

Modified Vetiver Oil: Economic Biopesticide

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Vetiver oil is obtained from *Vetiveria zinzanoides* L., a grass that can be found in both tropical and subtropical parts of the world. The roots of this grass on steam distillation yield an essential oil, mainly consisting of sesqiterpenes (3-4 %), sesquiterpenols (18-25%) and sesquiterpenones (7-8%). In this report we demonstrate that, sesquiterpenones and structurally related compounds exhibiting potent insecticidal activities can be enriched (35-50%) by simple chemical modification of vetiver oil. Initial studies and results utilizing modified vetiver oil for antifeedant and repellency against the Formosan subterranean termite, *Coptotermes formosanus* are discussed.

Terpenoids, especially monoterpenes and sesquiterpenes present in a number of plant-derived natural products and essential oils may have potential as insect repellents and feeding deterrents (*1,2*). Many of the insecticidal terpenes that have been tested are monoterpenoids, such as citral, eugenol, geraniol, nepetalactone, nerol, and thymol (*3-5*). However due to the limitations of bioavailability and volatility, use of monoterpenoids as biopesticides has been sparce. On the other hand there are limited reports of essential oil containing

complex mixtures of sesquiterpenes possessing insecticidal or repellent activity (*6,7*). Previous literature indicating insecticidal activity of sesquiterpenones was recently reviewed (*8,9*). Ibrahim et al. (*10*) also studied two derivatives of nootkatone, namely 1,10-dihydro- and tetrahydronootkatone, and found that they were all toxic to the Formosan subterranean termite, *Coptotermes formosanus*. Of these, tetrahydronootkatone exhibited the highest toxicity. However, to develop economical biopesticides based on a single compound or natural product components is difficult. In our approach we tried to integrate available knowledge on structure-activity relationships of sesquiterpenes in commercially available essential oils, and found that Vetiver oil was the best candidate available as it is enriched with 65% sesquiterpenes.

Vetiver oil is obtained by steam distillation of the aromatic roots from the tropical grass *Vetiveria zinzanoides*, mostly from Southeast Asia where it is also called Khus. Qualities of the refined oil include a characteristic 'greenish', 'precious-woody' odor with subtle grapefruit-like nuances. The oil contains a large number of oxygenated sesqiterpenes, the most important of which are shown in Figure 1. Lately, vetiver grass has gained focus for quite another reason. Its very excessive roots are effective in preventing soil erosion in the tropics where the original forests have been largely destroyed (*11*).

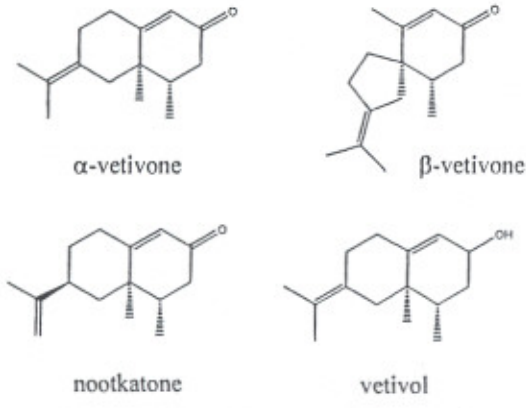

Figure 1. Sesquiterpenes in Vetiver oil

The major secondary class of sesquiterpenes present in Vetiver oil are biologically less potent (*12*) polar component vetivols or sesquiterpenols. These inactive sesquiterpene alcohols can be converted to ketones or sesquiterpenones or vetivones and khusinal by a simple oxidation processes without chromatographic separation.

Materials and Methods

Materials. Vetiver oil was purchased from Texarome, Inc. (Leakey, TX), and nootkatone (>97% crystalline) was purchased from Lancaster Synthesis (Windham, NH). Tetrahydronootkatone (95%) and modified vetiver oil mixtures were synthesized in our laboratory. Pyridinium dichromate (PDC) and palladium (Pd) used for the modification of vetiver oil and nootkatone were purchased from Aldrich Chem. Co. (Saint Louis, MO).

In the experimental approach for the modification of vetiver oil, we dissolved 10 g of vetiver oil in 100 ml of dry dichloromethane (DCM). To the resulting solution was added 1.25 weight equivalent of PDC and the mixture was stirred for 8 hr. DCM was evaporated on rotary evaporator and resulting crude mixture was redissolved in diethyl ether, filtered through a bed of celite and fluorosil (10 g). The filtered ethereal solution was concentrated on rotary evaporator and the resulting crude mixture was used for bioassay as PDC-oxidized vetiver oil. By following standard reaction procedures (*13*) 3 g of PDC oxidized vetiver oil was further modified by Pd/C catalyzed hydrogenation to generate saturated sesquiterpenones and sesquiterpenes. Proportions of sesquiterpenes, sesquiterpenones and sesquiterpenols were determined by gas chromatography (GC) and gas chromatography-mass spectroscopy (GC/MS) analyses (*12*). Simultaneously PDC-oxidation was performed on fractionated vetivols (polar fraction) of vetiver oil. Thus a total of eight compounds/mixtures were analyzed for their bioactivity in three different assays against workers of *C. formosanus* collected in standard bucket traps (*14*) set up in New Orleans, LA.

Choice Test Bioassay. We used 50 X 9 mm tight-fit Petri dishes (Falcon) with 2 filter paper disks (1 cm dia., 0.78 cm^2 area) attached at opposite ends of the dish with 1µl DCM (Figure 2). One of the 2 disks was treated with 1, 5, or 25 $\mu g/cm^2$ of a test compound in 10 µl ethanol. Two of the test compounds were also tested at 0.2 $\mu g/cm^2$. Control disks were treated with 10 µl ethanol. Each treatment dose was replicated 4 times. Five workers were released into each dish and distribution of termites recorded every 15 minutes for 5 hours under dim red light with a time lapse video recording system (Panasonic, Japan). The data were then transcribed and statistically analyzed (*15*).

Tunneling Assay. Ten grams of moist sand was placed in each of the two 30 ml plastic cups with lids (Bio-Serv, Frenchtown, NJ). The cups were connected with a 15 cm glass tube (4mm ID). The tube was filled with sand treated with 0, 5, 25 or 125 µg of test compounds per gram of sand (Figure 3). Each treatment dose was replicated 4 times. Fifty workers and 3 soldiers were released into one of the cups and tunneling into the tubes observed after 24, 30,

36, 42, 48, 54 and 72 hours. A test was terminated once termites tunneled all the way across the tube.

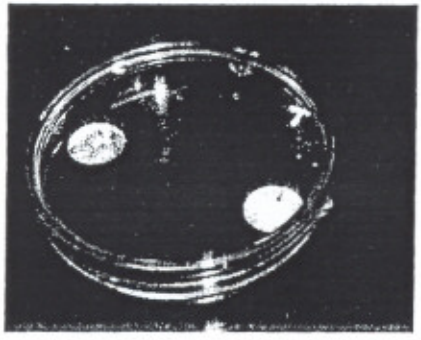

Figure 2. The setup for a choice test with C. formosanus *workers.*

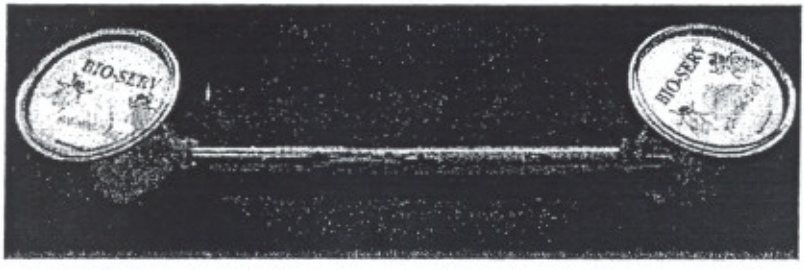

Figure 3. Experimental setup to determine tunneling through treated sand.

Feeding and Mortality. Filter paper disks (25 mm dia) were oven-dried and weighed. The test compounds were applied to the filter paper at 0, 1, 5, and 25µg/cm2. After allowing the solvent to evaporate, the filter paper was placed in a 50 X 9 mm Petri dish with a thin layer of moist sand. Termites (50 workers and 3 soldiers) were then released into the Petri dish and placed in an incubator maintained at 28^{0}C and 70% RH. After 5 days the unconsumed filter papers were removed, cleaned of debris, oven dried and weighed to determine the consumption. Live termites were counted to determine mortality. There were 4 replicates for each concentration of each chemical.

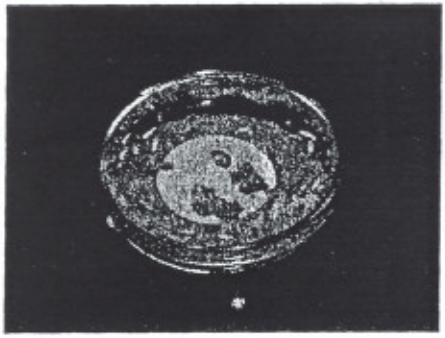

Figure 4. Feeding assay with C. formosanus *workers.*

Results and Discussion

Bioactivity of modified vetiver oil to Formosan subterranean termites is shown in Tables 1, 2, 3 and 4. Among the two valencenoids (bicyclic sesquiterpenes), vetiver oil and five modified vetiver oil mixtures tested, PDC-oxidized vetiver oil was the most repellent having consistent activity and a repellency threshold of 5 $\mu g/cm^2$ of treated filter paper.

Choice Test Results. PDC-oxidized vetiver oil was highly repellent even at 1 $\mu g/cm^2$ dose (Table 1). Nootkatone and tetrahydronootkatone were slightly repellent at a lower dose of 0.2 µg. At higher doses, these two compounds were either neutral or acted as attractants. Tetrahydronootkatone was however strongly repellent at 25 $\mu g/cm^2$.

Tunneling Results. At 0, 5, and 25 µg/g sand, the test chemicals did not affect tunneling activity and the termites tunneled through the tubes in all cases (Table 2). At 125 µg only 50% tubes with vetiver oil and PDC-oxidized vetivols were completely tunneled. For tubes treated with nootkatone and polar fraction of vetivols only 75% were completely tunneled. In case of vetiver oil, for those termites that completely crossed the tubes, it took an average of 24 hours. However, for nootkatone average time to cross the tube was 160 hours.

Feeding and Mortality Results. None of the treatments at 1 and 5 $\mu g/cm^2$ of filter paper aused a significant decrease in feeding (Table 3). At 25 $\mu g/cm^2$ PDC- oxidized vetiver oil vetivols resulted in a significant reduction in feeding. Tetrahydronootkatone caused the highest mortality at 25 $\mu g/cm^2$ dose, followed by nootkatone, vetiver oil and Pd/C reduced-oxidized vetiver oil (Table 4). None of the compounds caused any significant mortality at lower doses. Overall, tetrahydronootkatone appeared to be the best treatment.

Table 1. Distribution of *C. formosanus* workers on treated vs. control filter paper disks treated with various test chemicals at three concentrations in a choice test.*

	Treatment	% Termites on control and treated disks			
			1 μg/cm^2	5 μg/cm^2	25μg/cm^2
1	Nootkatone	control	17.8 ± 20.6	19.9 ± 6.3	8.1 ± 3.4
		treatment	28.5 ± 25.3	7.6 ± 5.4	18.3 ± 2.7
2	Tetrahydronootkatone	control	4.0 ± 0.8	20.4 ± 8.2	16.4 ± 2.2
		treatment	15.0 ± 12.0	10.7 ± 1.7	10.5 ± 0.3
3	Vetiver oil	control	24.0 ± 7.5	23.0 ± 8.6	4.5 ± 4.2
		treatment	4.5 ± 3.3	8.8 ± 2.5	10.4 ± 0.5
4	Non-polar fr.	control	15.9 ± 7.0	13.1 ± 9.9	23.3 ± 5.1
		treatment	25.9 ± 10.2	22.9 ± 10.5	14.8 ± 7.0
5	Polar fr. (vetivols)	control	11.2 ± 3.1	30.2 ± 4.0	22.9 ± 13.7
		treatment	22.9 ± 8.5	7.4 ± 3.5	9.3 ± 3.5
6	PDC oxidized vetiver oil	control	31.0 ± 8.9	31.7 ± 9.0	24.5 ± 4.8
		treatment	2.1 ± 0.9	5.9 ± 2.4	2.1 ± 1.0
7	Pd/C reduced oxi. Vetiver oil	control	21.9 ± 5.4	13.6 ± 6.3	20.7 ± 3.7
		treatment	16.9 ± 8.4	28.1 ± 6.8	25.7 ± 9.6
8	PDC oxidized vetivols	control	13.1 ± 9.6	7.9 ± 4.7	6.2 ± 2.8
		treatment	11.2 ± 9.0	8.1 ± 3.1	7.1 ± 6.2

* Values are averages ± SE, N = 4.

Table 2. Tunneling and time to tunnel (hours) by *C. formosanus* workers through sand treated with various test chemicals at three concentrations.*

	Treatment	% Crossed at dose (µg/g)			
		0	5	25	125
1	Nootkatone	100	100	100	75
2	Tetrahydronootkatone	100	100	100	100
3	Vetiver oil	100	100	100	50
4	Non-polar fr.	100	100	100	100
5	Polar fr. (vetivols)	100	100	100	75
6	PDC oxidized vetiver oil	100	100	100	100
7	Pd/C reduced oxi. Vetiver oil	100	100	100	100
8	PDC oxidized vetivols	100	100	100	50

	Treatment	Average hours taken to cross at dose (µg/g)			
		0	5	25	125
1	Nootkatone	24 ± 0.0	24 ± 0.0	24 ± 0.0	160 ± 27.7
2	Tetrahydronootkatone	30 ± 6.0	30 ± 6.0	24 ± 0.0	78 ± 6.0
3	Vetiver oil	24 ± 0.0	24 ± 0.0	24 ± 0.0	24 ± 0.0
4	Non-polar fr.	30 ± 6.0	24 ± 0.0	24 ± 0.0	24 ± 0.0
5	Polar fr. (vetivols)	42 ± 11.5	36 ± 6.9	54 ± 22.7	40 ± 6.9
6	PDC oxidized vetiver oil	24 ± 0.0	24 ± 0.0	36 ± 6.9	24 ± 0.0
7	Pd/C reduced oxi. Vetiver oil	24 ± 0.0	24 ± 0.0	30 ± 6.0	24 ± 0.0
8	PDC oxidized vetivols	24 ± 0.0	72 ± 21.9	48 ± 13.8	24 ± 0.0

*Values are averages ± SE, N = 4.

Table 3. Consumption (mg) by *C. formosanus* workers of filter paper treated with various test chemicals at three different concentrations.*

	Treatment	Average feeding (mg)			
		Control	1 µg/cm^2	5 µg/cm^2	25 µg/cm^2
1	Nootkatone	22.7 ± 2.4	22.7 ± 2.7	13.3 ± 2.3	19.3 ± 1.8
2	Tetrahydronootkatone	15.7 ± 3.1	24.7 ± 3.6	13.9 ± 3.1	10.1 ± 3.0
3	Vetiver oil	18.8 ± 2.3	18.9 ± 3.7	20.5 ± 3.3	20.0 ± 3.1
4	Non-polar fr.	18.2 ± 2.9	16.0 ± 1.0	21.1 ± 2.0	12.9 + 2.4
5	Polar fr. (vetivols)	19.5 ± 4.2	16.1 ± 2.3	18.2 ± 5.4	15.7 ± 3.0
6	PDC oxidized vetiver oil	22.3 ± 2.0	17.1 ± 3.8	16.1 ± 3.5	10.1 ± 1.8
7	Pd/C reduced oxi. Vetiver oil	19.1 ± 1.9	14.5 ± 2.8	19.3 ± 3.1	17.8 ± 2.0
8	PDC oxidized vetivols	11.8 ± 4.1	17.2 ± 3.1	15.7 ± 4.6	9.1 ± 2.1

Table 4. Mortality of *C. formosanus* workers caused by exposure to/feeding on filter paper with test chemicals at three different concentrations.*

	Treatment	Average mortality			
		Control	1 μg/cm²	5 μg/cm²	25 μg/cm²
1	Nootkatone	8.2 ± .0	4.6 ± 1.6	6.4 ± 3.4	20.0 ± 4.4
2	Tetrahydronootkatone	2.7 ± 2.0	6.4 ± 2.1	5.5 ± 2.9	30.0 ± 4.9
3	Vetiver oil	5.4 ± 1.9	0.9 ± 1.0	6.4 ± 3.0	10.0 ± 3.3
4	Non-polar fr.	4.5 ± 2.5	3.7 ± 1.9	1.8 ± 1.2	4.5 ± 3.2
5	Polar fr. (vetivols)	4.6 ± 2.7	2.8 ± 1.2	5.7 ± 1.0	1.8 ± 1.2
6	PDC oxidized vetiver oil	2.8 ± 1.2	4.6 ± 1.6	5.5 ± 1.9	5.5 ± 1.9
7	Pd/C reduced oxi. Vetiver oil	4.6 ± 1.6	2.8 ± 1.2	5.5 ± 1.0	10.0 + 1.9
8	PDC oxidized vetivols	2.7 ± 3.0	3.6 ± 4.0	2.8 ± 1.2	3.7 ± 2.0

*Values are averages ± SE, N = 4.

Summary

Modified vetiver oil, enriched with sesquiterpenones (PDC-oxidized) exhibited potent repellency to Formosan subterranean termites at different concentrations in choice test while tetrahydronootkatone exhibited significant repellency at only the higher dose. Tetrahydronootkatone caused the highest mortality at 25 μg/cm² dose, followed by nootkatone, vetiver oil and Pd/C reduced-oxidized vetiver oil. Overall tetrahydro nootkatone appeared to be the best treatment in feeding and mortality experiments. At 125 μg dose only 50% tubes with vetiver oil and PDC oxidized vetivols were completely tunneled. At 125 μg dose 75% tubes treated with nootkatone and vetivols were completely tunneled. At the same dose, for the nootkatone the average time to cross the tube was 160 hrs. Sesquiterpenoids known in nature outnumber monoterpenoids (*16*) and have low mammalian toxicity. The most important advantage for sesquiterpenoid as repellents is their persistence and low volatility (*17*).

By simple modifications we can enhance desired bioactivities of natural product extracts or essential oils without isolating individual components. In the case of vetiver oil, the insect repellent or deterrent activity is due to the presence of sequiterpenones (cyclic ketones); we can enrich these sequiterpenones by oxidizing relatively inactive sesquiterpenols (cyclic alcohols). Thus modified vetiver oil, enriched with sequiterpenones would be a product of an economical and environmentally sound bio-pesticide.

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